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Supplementary Information

Integrating resonance energy transfer with enzyme-triggered hydrolysis for ultrasensitive electrochemiluminescence detection of exosomes

Yuanyuan Wang[a, b], Xianwen Kan [a, *]

[a] Key Laboratory of Functional Molecular Solids, Ministry of Education, Anhui Laboratory of Molecule-Based Materials, Anhui Key Laboratory of Chemo-Biosensing, College of Chemistry and Materials Science, Anhui Normal University, Wuhu 241000, PR China

[b] Scholl of Basic Courses, Bengbu Medical College, Bengbu 233030, PR China

*Corresponding author:

Xianwen Kan

E-mail: kanxw@mail.ahnu.edu.cn;

Tel: +86-553-3937135;

Fax: +86-553-3869303.

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S1. Experimental section.

Materials and reagents. $ZrCl_4$, terephthalic acid (BDC), 2-amino-terephthalic acid (BDC-NH_2), 1,1,2,2-Tetra (4-carboxy phenyl) ethylene (TPE), ammonium persulphateand (APS), and tetramethylethylene-diamine (TEMED) were obtained from Aladdin Biochemical Technology Co., Ltd. 2-(N-morpholino) ethanesulfonic acid (MES) buffer, L-ascorbic acid 2-phosphate (AAP), and potassium permanganate ($KMnO_4$) were supplied by Macklin Biochemical Co., Ltd. Biotin-EpCAM aptamer, complementary DNA 1, 2, 3 (C1, C2, and C3) (corresponding sequences was displayed in Table S2), DNA Marker A (25~500 bp), streptavidin-alkaline phosphatase (S-ALP), biotin solution, streptavidin-magnetic beads (S-MBs), TBS buffer (containing 50 mM Tris-HCl and 150 mM NaCl, pH=7.4), TBST buffer (containing 50 mM Tris-HCl, 0.05 M NaCl, 0.02% Tween-20, pH 7.4), Acrylamide/N,N'-methylenediacrylamide 30% Solution (29:1), and 5×TBE buffer (containing 445 mM Tris, 445 mM Boric acid and 10 mM EDTA, pH 8.0-8.6) were purchased from Songon Biotech. Co., Ltd. A549 cells and Hela cells were obtained from Beyotime Biotech. Co., Ltd. UltroPower DNA stain was purchased from Bio Teke Co., Ltd. Dulbecco's modified Eagle medium (DMEM), Roswell Park Memorial Institute (RPMI)-1640 medium and fetal bovine serum (FBS), and 6×orange DNA loading dye were acquired from ThermoFisher Scientific Co., Ltd. All other chemicals were of analytical grade and double distilled water was used to prepare aqueous solutions.

Apparatus. Scanning electron microscopy (SEM) images and energy dispersive X-ray spectroscopy (EDX) spectrum were observed on an S-8100 microscope (Hitachi Co., Ltd, Japan). Transmission electron microscopy (TEM) images were obtained on an HT-7700 field emission transmission electron microscope (Hitachi Co., Ltd, Japan). Scanning transmission electron microscopy (STEM) image and elemental mapping were measured on an FEI Talos F200X field emission transmission electron microscope (Thermo Fisher Co., Ltd, USA). Powder X-ray diffraction (XRD) analysis was performed on a Smartlab Advance X-ray diffractometer (Rigakur Co., Ltd, Japan). UV-vis absorption spectrum was carried out on a U-2910 spectrophotometer (Hitachi

Co., Ltd, Japan), Fourier transform infrared spectroscopy (FT-IR) was measured using an FT-IR-8400S spectrometer (Shimadzu Co., Ltd, Japan), X-ray photoelectron spectroscopy (XPS) spectra was obtained on a K-Alpha spectrometer (Thermo Scientific, the USA). Zeta potential was detected on a ZSE nanoparticle potentiometric analyzer (Malvern, Britain). Brunauer Emmett Teller (BET) analysis was accomplished on a Micromeritics Instrument ASAP 2460 (Micromeritics Instrument, the USA). Photoluminescence spectra was analyzed by an F-4700 fluorescence spectrophotometer (Hitachi, Japan). Gel imaging was performed on a Bio-image system (Fusion FX, France). ECL measurements were performed on an MPI-E II electrochemiluminescence analyzer (Xi'an Remax, China). Electrochemical experiments were measured on a CHI 660E electrochemical workstation (Shanghai Chenhua, China). Three-electrode system containing a glassy carbon electrode (GCE, Φ=3 mm) as the working electrode, an Ag/AgCl (3M KCl solution) as the reference electrode, and a platinum wire as the counter electrode was used through the electrochemical and ECL detection procedure.

Synthesize of TPE-UIO-66@MnO_2.

Firstly, TPE-UIO-66 was synthesized by the hydrothermal method in one step.[1] 0.077 g of $ZrCl_4$, 0.060 g of terephthalic acid (BDC), 0.048 g of 2-amino-terephthalic acid (BDC-NH_2), and 0.0023 g of 1,1,2,2-Tetra (4-carboxy phenyl) ethylene (TPE) were dissolved in 6 mL of N, N-dimethylformamide (DMF), followed by adding 60 μL of HCl. The resulted solution was heated to 120 °C in a Teflon-lined stainless-steel autoclave for 48 h. Finally, the pale-yellow precipitate was obtained by centrifugation. After washing with water for several times, the product was dried at 60 °C overnight and stored at room temperature.

TPE-UIO-66@MnO_2 was obtained via in situ synthesis of MnO_2 nanosheets on the surface of the TPE-UIO-66. Briefly, 1.0 mg/mL of TPE-UIO-66 was dispersed in 2-(N-morpholino) ethanesulfonic acid (MES) buffer (0.1 M, pH 6.0). Then, 2 mM of $KMnO_4$ was added and the mixture was ultrasonic for 30 min. The obtained product was washed with water for three times and redispersed in pure water for later use.

Cell culture and exosome extraction.

A549 cells and Hela cells were cultured in a 37 °C incubator with 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) and Roswell Park Memorial Institute (RPMI)-1640 medium, respectively. And both the mediums were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. After the cells were grown to 80% confluence, the medium was changed by that containing 10% exosome-depleted FBS for 48 h. Then, the medium was collected and centrifuged at 3000 g for 10 min to remove dead cells and cell debris. The supernatant was collected and then stored at -80 °C for further use. The exosomes were extracted by adding 2.5 mL of the Hieff® Quick exosome isolation kit (for cell culture media) to 10 mL of the supernatant and vortex mixing for 1 min before staying at 4 °C for 2 h. After centrifuging at 10000 g for 1 h at 4 °C, the precipitate was resuspended in PBS and further centrifuged at 12000 g for 2 min at 4 °C, the resulted supernatant was kept at -80 °C until use.

Characterization of the exosomes.

TEM characterization. TEM images were obtained using Negative staining method with some modifications.[2] Briefly, 4 μL of the exosome suspension was dropped on the Copper grid for 1 min, followed by washed two times with 10 μL of 1% Phosphotungstic acid solution. Later, 10 μL of 1% Phosphotungstic acid was dropped on the Copper mesh for 1 min and dried in the air, and the morphology of exosomes was imaged on a TEM at an acceleration voltage of 100 kV.

NTA analysis. The separated exosome suspension was diluted 100-fold with PBS and filtrated with 0.22 μM microporous membrane, then the diameter and concentration were measured on a NanoSight NS300 nanoparticle tracking analyzer (Malvern Panalytical, China).

Western blot assay. Western blot assay was performed according to previous study with a slight modification.[3] Firstly, the exosomes proteins were extracted under repeated freeze-thaw operations, followed by separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis at 80 V. Next, they were subjected to membrane transfer operation at 250 mA for 1 h, and then blocked by 5% skimmed milk powder for 1 h. The blocked membrane was allowed to incubate with primary

antibodies including EpCAM and CD63 overnight, and then incubated with secondary antibodies for 1 h. Finally, the membrane was imaged on the gel imaging system.

Fabrication of the exosome-initiate enzyme-triggered ECL-RET sensor.

Prior to the construction of the sensor, the EpCAM aptamer-magnetic beads (MBs) bioconjugations that can specifical bind exosomes with high expression of EpCAM were prepared by an affinity reaction between streptomycin and biotin. Briefly, 100 μL of the streptomycin-modified MBs (10 mg/mL, Fig. S1) were separated by the magnet and washed three times with TBST buffer (50 mM Tris-HCl, 0.05 M NaCl, 0.02% Tween-20, pH 7.4). Then they were suspended to 1 mL of 1 μM and gently rolled for 30 min before being washed three times with 1 mL of TBST. After magnetic separation, the aptamer-MBs were blocked by redispersing in 1 mL of 400 μM biotin and reacted for 30 min with gentle rolling, followed by being washed with TBST and resuspended in the mixing solution of C1, C2 and C3 (300 μL, 3.33 μM for each cDNA). The mixture was allowed to stay for 2 h at 37 °C. After that, the three oligonucleotides (C1, C2, and C3) partially complementary with EpCAM aptamer were successfully hybridized on the aptamer-MBs bioconjugations. After washing three times with TBS buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4), the C1/C2/C3- aptamer-MBs were dispersed in 1 mL of 10 μg/mL streptavidin-alkaline phosphatase and reacted for 30 min with gentle shaking. In this step, streptavidin-ALP was bind with the bition-C1/C2/C3 on the aptamer-MBs bioconjugations through affinity between streptomycin and biotin. The final ALP-C1/C2/C3-aptamer-MBs were washed three times with TBS buffer, then suspended in 1 mL of TBS buffer and stored at 4 °C for further use.

To fabricate the sensor, 10 μL of various concentrations of exosomes were co-incubated with 50 μL of the aptamer-C1/C2/C3-ALP-MBs for 2 h at 37 °C to release the C1/C2/C3-ALP, followed by magnetic separation. Then 40 μL of the supernatant was collected and incubated with 20 μL of 10 mM AAP for 30 min at 37 °C. Subsequently, 20 μL of the TPE-UIO-66@MnO_2 dispersion was added and reacted for 10 min at room temperature. Finally, 6 μL of the mixture was dropped onto the cleaned GCE before dried.

ECL detection of the proposed sensor.

This sensor was measured in 50 mM TBS buffer (pH 7.4) containing 0.1 M $K_2S_2O_8$. ECL detection was performed under cyclic voltammetry sweeping from 0 V to -2 V, the scan rate was set as 0.1 V/s and the photomultiplier tube (PMT) was set at 700 V. ECL-λ spectra were obtained by collecting the ECL signals during cyclic potential sweep from 0 to -2 V with different optical filters (400 nm, 412 nm, 425 nm, 440 nm, 460 nm, 475 nm, 490 nm, 505 nm, 520 nm, 535 nm, 555 nm, 575 nm, 590 nm, 605 nm, 620 nm).

Application of the ECL sensor in real biosamples.

Human serum samples from healthy people and lung cancer patients were collected and centrifuged at 3000 g for 10 min. Subsequently, the serum was transferred to a new EP tube and centrifuged at 10000 g for 20 min. Then, 400 μL of the supernatant was collected and diluted with 1.6 mL of PBS (10 mM, pH 7.4), followed by adding 400 μL of the Hieff® Quick exosome isolation kit (for human serum) and allowed to react for two hours at 4 °C. After centrifugation for 1 h at 4 °C (10000 g), the precipitate was resuspended in 160 μL PBS and centrifuged for 2 min at 4 °C (12000 g), the supernatant was stored at -80 °C for later use. 10 μL of the sample was used as analyte in the sensor. All experiments were accomplished under the approval of the Ethics Committee of Anhui Normal University.

Polyacrylamide gel electrophoresis (PAGE) analysis.

10% polyacrylamide gel was prepared by mixing 8.3 mL of acrylamide/N, N'-methylenediacrylamide 30% Solution, 5 mL of 5×TBE buffer, 11.5 mL of H_2O, 200 μL of 10% APS, and 25 μL of TEMED, followed by polymerizing for 16 min at 37 °C. The loading sample was prepared by mixing 7 μL of DNA chains or their hybridization reaction products with 1.5 μL of DNA stain and 1.5 μL of 6×orange DNA loading dye. Gel electrophoresis was performed at 100 V for 120 min in 1×TBE

buffer using a DYY-6C electrophoresis apparatus (Beijing LIUYI, China) and imaged on the imaging system equipped with UV light.

Calculation of the detection limit.

Detection limit, expressed as the concentration, c_L, is derived from the smallest measure, x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is given by the equation $x_L = x_{b1} + 3s_{b1}$, where x_{b1} and s_{b1} represent the mean of the blank measures and the standard deviation of the blank measurements, respectively.[4]

In this work, after making twenty measurements of blank, the x_{b1} and s_{b1} were calculated to be 1765.4 and 67.1, respectively. Therefore, $x_L = x_{b1} + 3s_{b1}$= 1765.4+3×67.1=1966.7. The calculation plot of the proposed sensor was $I = -1345.8 + 1073.3lgc$. Then, the $c_L = 10^{(1966.7+1345.8)/1073.3}$=1.22×10^3 particles/mL. Therefore, LOD was 1.22×10^3 particles/mL.

S2. Supplementary Figures and Tables.

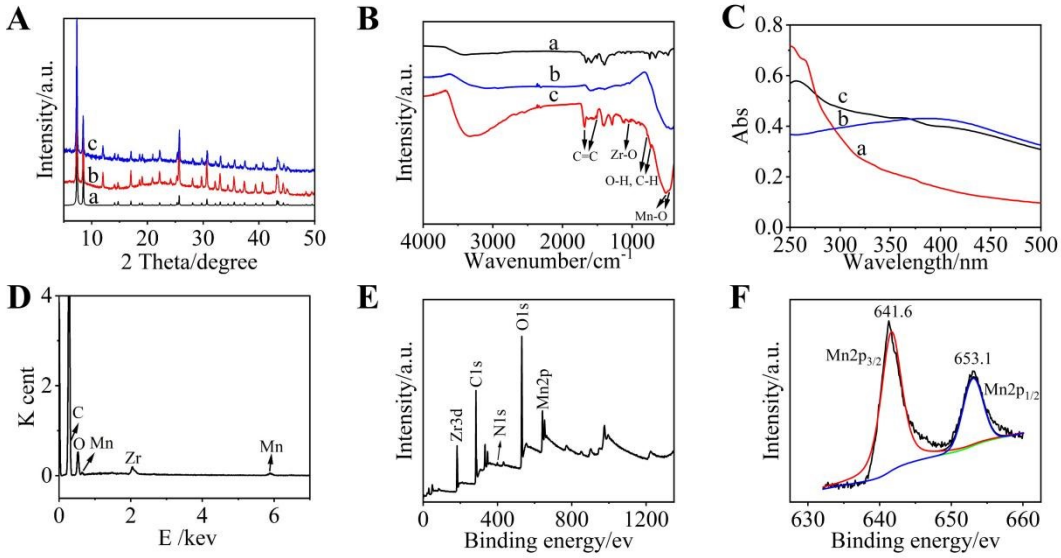

Fig. S1 (A) XRD patterns of simulated UIO-66 (a), TPE-UIO-66 (b), and TPE-UIO-66@MnO_2 (c). (B) FTIR spectra and (C) UV-visible absorption spectra of TPE-UIO-66 (a), MnO_2 (b), and TPE-UIO-66@MnO_2 (c). (D) EDX spectra of TPE-UIO-66@MnO_2. (E and F) XPS spectra of TPE-UIO-66@MnO_2 (E) the Mn element (F).

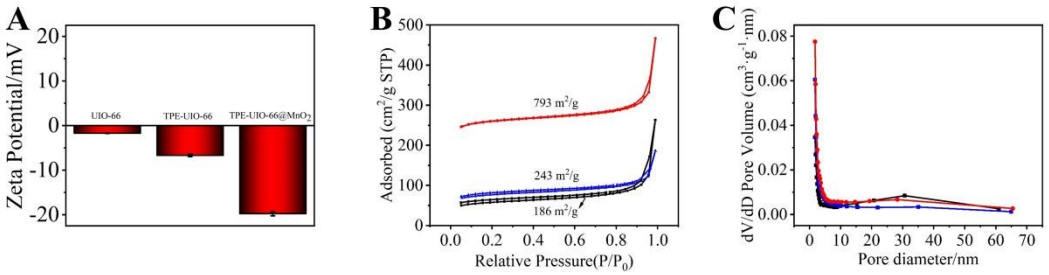

Fig. S2 (A) Zeta potential, (B) Nitrogen adsorption-desorption isotherm, and (C) DFT pore size distribution of UIO-66 (a), TPE-UIO-66 (b), and TPE-UIO-66@MnO_2 (c). (Error bars=standard deviations, n=3).

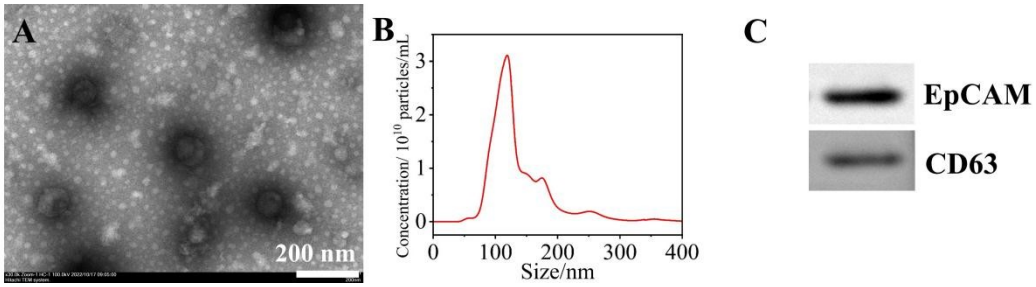

Fig. S3 (A) TEM image, (B) NTA analysis, and (C) Western blot analysis of the exosomes derived from A549 cells.

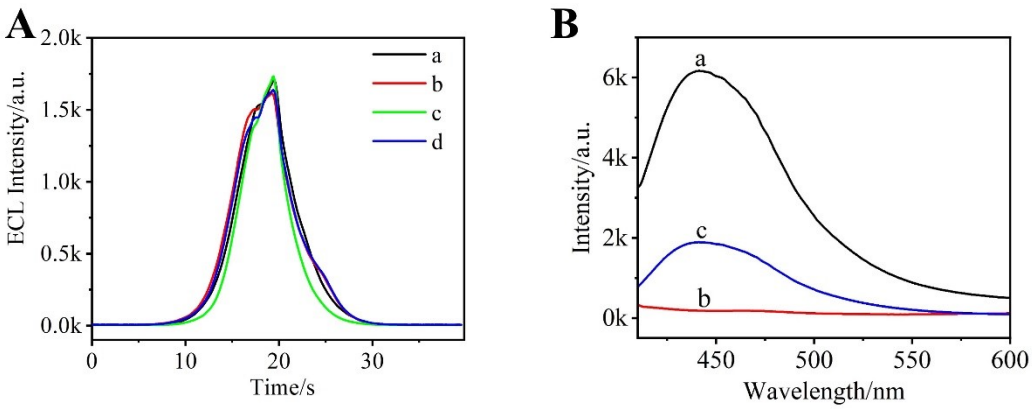

Fig. S4 (A) ECL responses of the sensor for blank (a), 6 μL mixtures of (40 μL Tris-HCl buffer + 20 μL 2 mM $MnCl_2$ + 20 μL TPE-UIO-66@MnO_2)/GCE (b), (40 μL Tris-HCl buffer + 20 μL 10 mM AAP + 20 μL TPE-UIO-66@MnO_2)/GCE (c), and (40 μL C1/C2/C3 (1 μM)-ALP (10 μg/mL) + 20 μL Tris-HCl buffer + 20 μL TPE-UIO-66@MnO_2)/GCE (d). (B) Photoluminescence (PL) emission spectrum of TPE-UIO-66 (a), TPE-UIO-66@MnO_2 (b), and the mixture of TPE-UIO-66 and MnO_2 (c).

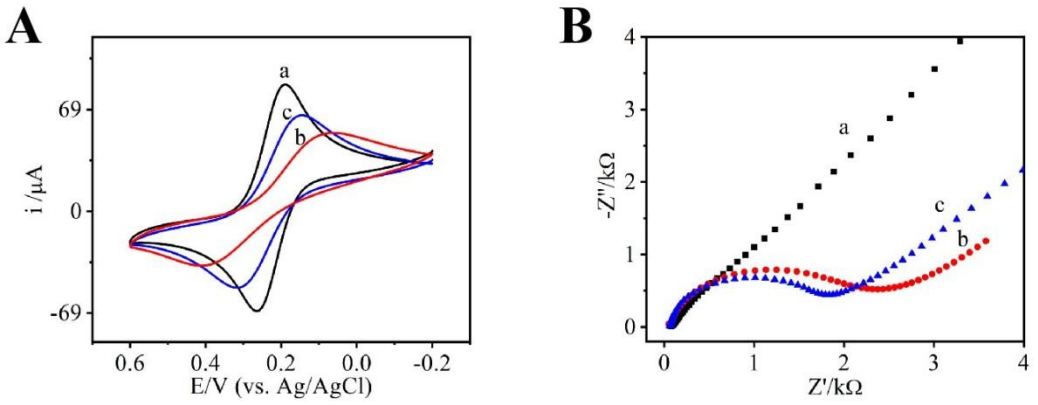

Fig. S5 (A) CV and EIS assays for bare GCE (a) and the modified sensor for blank (b) and exosomes (c) in 0.1 M KCl solution containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$.

A peak-to-peak potential separation (ΔEp) of 75 mV was recorded on the bare GCE, while that of the sensor for blank was 340 mV, owing to the poor conductivity of TPE-UIO-66@MnO_2. And ΔEp recorded on the sensor for exosomes (172 mV) was lower than that of the blank, which may attribute to the decreased content of nearly non-conductive MnO_2. Additionally, EIS measurement showed similar tendency.

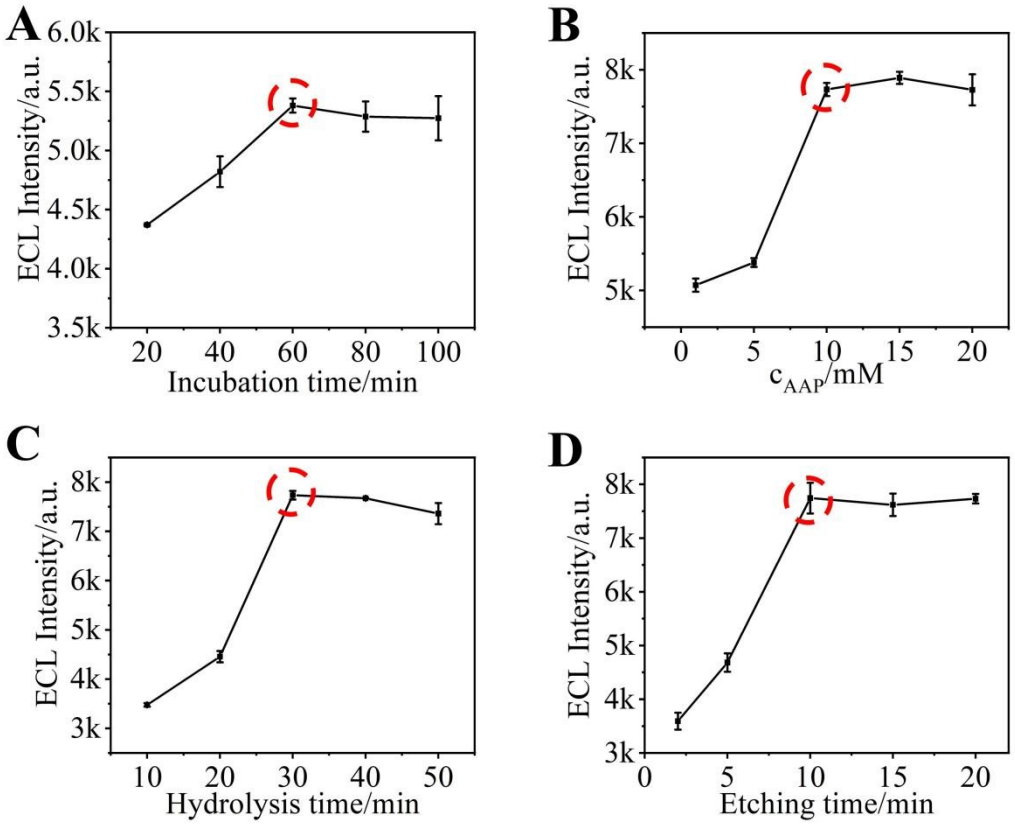

Fig. S6 Effect of the incubation time of exosome (A), the concentration of the AAP (B), the hydrolysis time of the AAP (C), and the etching time for MnO_2 (D) on the ECL intensity of the sensor for exosome with a concentration of 3.24×10^8 particles/mL. (Error bars=standard deviations, n=3).

The optimal experimental conditions including the incubation time of exosome, the concentration and the hydrolysis time of AAP, the etching time for MnO_2 were explored. As illustrated in Fig. S6A, the ECL intensity increased with the incubation time of exosomes from 20 min to 60 min, and maintaining relatively stable above 60 min. Therefore, 60 min was selected as the optimal incubation time of exosomes. To obtain the best analytical performance, AAP concentration with 2, 5, 10, 15, 20 mM were accessed. As shown in Fig. S6B, the ECL intensity gradually increased and stabilized at 10 mM. As a result, 10 mM was chosen as the optimal concentration of AAP. Similar tendency occurred on the optimal hydrolysis time observation (Fig. S6C), the best hydrolysis time for AAP was set at 30 min. Fig. S6D showed that the ECL intensity grew to the top when the etching time was set at 10 min. Thus, the optimized etching time was 10 min.

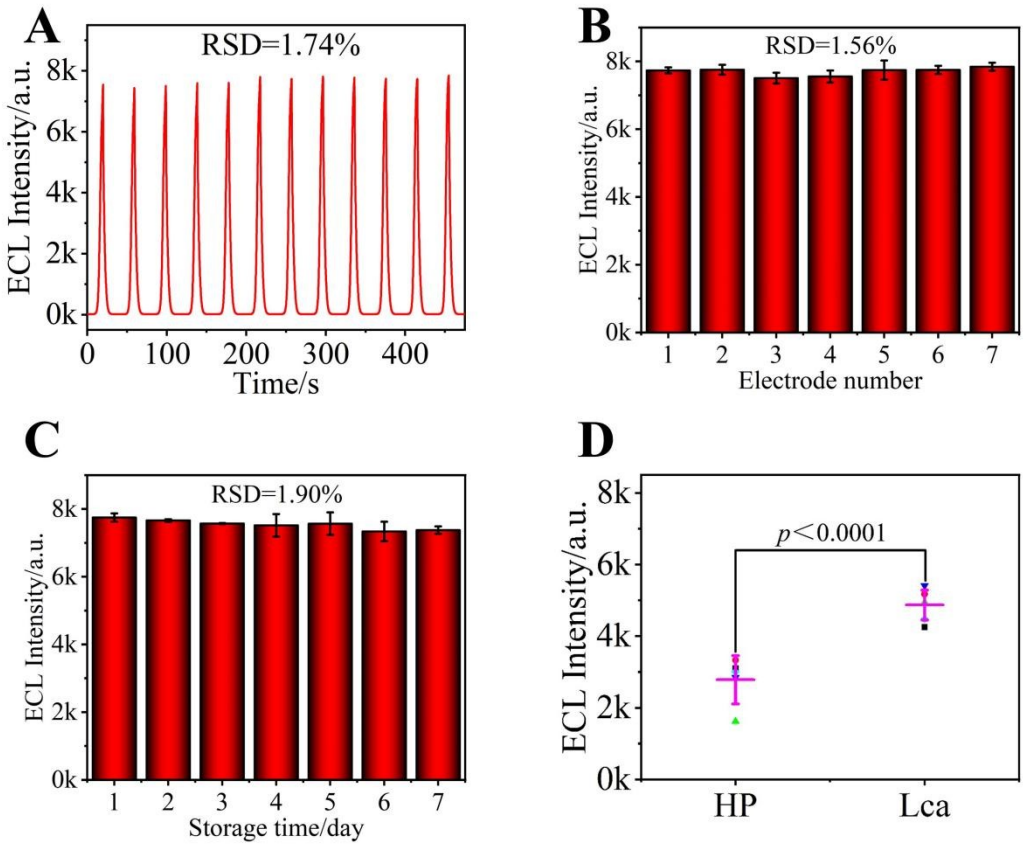

Fig. S7 (A) ECL-time spectra of the sensor for exosomes of 3.24×10^8 particles/mL under continuous scanning. (B) Repeatability and (C) stability assay of the proposed sensor for exosome with a concentration of 3.24×10^8 particles/mL. (Error bars=standard deviations, n=3). (D) ECL intensity of the sensor for exosomes from the serum of healthy person and lung cancer patients. (Error bars refer to standard deviations of at least three repeated measurements).

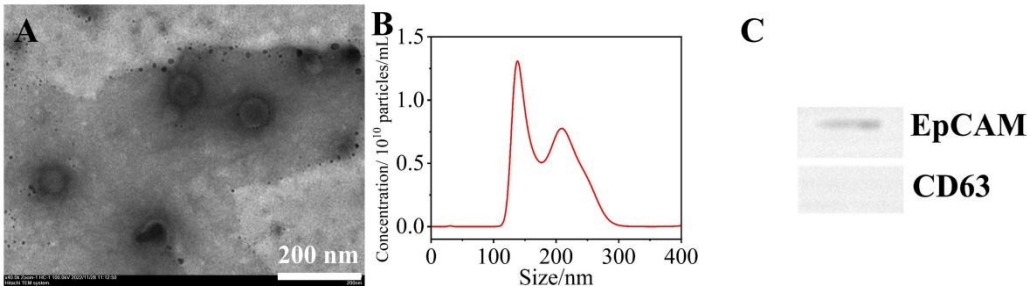

Fig. S8 (A) TEM image, (B) NTA analysis, and (C) Western blot characterization of the exosomes derived from Hela cells.

As shown in Fig. S8A, the exosomes derived from Hela cells displayed a cup-

shape under TEM characterization. NTA assay illustrated the concentration of the suspension was 1.32×10^{10} particles/mL, with an average diameter of about 180 nm (Fig. S8B). Fig. S8C depicted the low protein expression of EpCAM and CD63 on the exosomes compared to that derived from A549 cells.

Table S1. Comparison of analytical performances for exosomes detection with other sensors.

Method	Liner Range/ particles·mL^{-1}	Detection limit /particles·mL^{-1}	References
NiFe-Ru(bpy)$_3^{2+}$ modified aptasensor	2.3×10^4~2.3×10^9	5.0×10^3	5
CdS QDs@$CaCO_3$ microparticles modified GCE	5×10^4~1×10^8	2.1×10^4	6
luminol-AuNPs@g-C_3N_4 nanoprobe amplification	1.0×10^5~1.0×10^{10}	3.9×10^4	7
Ti_3C_2 MXenes nanosheets aptasensor	5×10^5~5×10^{11}	1.25×10^4	8
MXenes-BPQDs dual-mode probe	1.1×10^5~1.1×10^{11}	3.7×10^4	9
G-quadruplex/hemin DNAzyme aptasensor	3.4×10^5~1.7×10^8	7.41×10^4	10
Fluorescence	8.5×10^3~8.5×10^5	4.5×10^3	11
Colorimetric	1.0×10^3~1.0×10^{11}	7.7×10^3	12
Electrochemistry	3.4×10^3~3.4×10^8	3.4×10^3	13
TPE-UIO-66@MnO_2 ECL-RET aptasensor	1.62×10^4~1.62×10^9	1.22×10^3	This work

Note: reference from 5 to 10 were based on ECL method.

Table S2. DNA sequences used in the proposed sensor.

Name	Sequence (5'-3')
EpCAM aptamer	biotin-TTTTTTCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTG
C1	biotin-TTTTTGCCAACCCCCCA
C2	biotin-TTTTTCAACGTGGGACA

C3	biotin-TTTTTGCAACCTCTGTA

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